Immunological memory protective immunity

Zinkernagel, Rolf M

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MULTI-AUTHOR REVIEW

Immunological memory ≠ protective immunity

Rolf M. Zinkernagel

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Abstract So-called 'immunological memory' is, in my view, a typical example where a field of enquiry, i.e. to understand long-term protection to survive reexposure to infection, has been overtaken by 'l'art pour l'art' of 'basic immunology'. The aim of this critical review is to point out some key differences between academic text book-defined immunological memory and protective immunity as viewed from a co-evolutionary point of view, both from the host and the infectious agents. A key conclusion is that 'immunological memory' of course exists, but only in particular experimental laboratory models measuring 'quicker and better' responses after an earlier immunization. These often do correlate with, but are not the key mechanisms of, protection. Protection depends on pre-existing neutralizing antibodies or pre-activated T cells at the time of infection—as documented by the importance of maternal antibodies around birth for survival of the offspring. Importantly, both high levels of antibodies and of activated T cells are antigen driven. This conclusion has serious implications for our thinking about vaccines and maintaining a level of protection in the population to deal with old and new infectious diseases.

Keywords Antibodies · Antigen driven · Maternal antibodies · Acute lethal infection · Non-cytopathic persistent infections

R. M. Zinkernagel (✉)
University of Zurich, 8091 Zurich, Switzerland
e-mail: rolf.zinkernagel@pty.usz.ch

Introduction

In the beginning was the fact that, once recovered from an infection, the patient was resistant for life to disease by the same infection. This, we call immunity. But since the 1880s, with the beginning of modern immunology, the term immunological memory was borrowed from 'conventional' (neurological) memory to seemingly explain this fact. We still do not understand whether 'conventional' brain memory is due to once perceived or thought = always remembered, versus repeatedly reencountered, recollected, or dreamed. This controversy is the subject of this review on academic immunological memory versus immunity.

Immunological memory, specificity, and tolerance are three key parameters that immunologists study. Many have reviewed some aspects of this crucial triad of immunity and have voiced serious concerns, not only about the use of these words but also about their implied meaning [1–7]. Immunological memory is defined in text books as follows [2, 3]: 'The ability of the immune system to respond more rapidly and effectively to pathogens that have been encountered previously, and that reflects the pre-existence of clonally expanded populations of antigen specific lymphocytes. Memory responses which are called secondary depending on the number of exposures to antigens also differ qualitatively from primary responses. This is particularly clear in the case of the antibody response, where the characteristics of antibodies produced in secondary and subsequent responses are distinct. Specific memory is maintained by distinct populations of long-lived memory cells, that can persist without antigen' [2]. Another definition states [3]: 'Once the immune system has recognized and responded to an antigen, it exhibits immunological memory'. A second encounter with the same antigen

induces a heightened state of immune reactivity. Because of this attribute, the immune system can confer life-long immunity to many infectious agents after an initial encounter'. The alternative interpretation, that lymphocytes are repeatedly stimulated by antigen re-exposure within the host or from the outside, appears to have been settled in favor of the former type in most immunologists' mind [1–6]). The key question is, however, whether this 'immunological memory' (i.e. accelerated and heightened response) is good enough to protect the host from either new acutely lethal infections or re-infections for better survival of the species? There is no doubt about experimental documentation of academically defined immunological memory. If a mouse is primed with sheep red blood cells, then a second injection of red cells will reveal an accelerated and heightened response. This also applies to bovine serum albumin, nuclear protein of virus, or classical carrier hapten molecules [1–6]. While accelerated and better responses often correlate with protective immunity, they are alone definitely not sufficient [7–9]. The pre-existing titer of protective antibody or activity of highly activated effector T cells at the time of infection are generally accepted to determine whether or not a host is protected, or can control, an infection better than a naïve host [8, 10, 11]. In addition, it must be kept in mind that so-called innate or natural immunity is a very important basis for resistance to infection; this includes interferon, Toll-like receptor mediated effects, and so-called natural (non-intentionally) induced antibodies in serum [12]. The latter are highly specific protective antibodies as defined by neutralization assays and by protection. These natural antibodies simply reflect the genetically determined, existing spectrum of the specific B cell repertoire. This includes serotypically defined viral-, bacterial-, or toxin specificities. By definition, such natural antibodies are not cross-reactive but are serotypically specific as formally documented for neutralizing antibodies against viruses [12].

Why should higher vertebrates need 'immunological memory', be it for B cells or T cells? I have been arguing repeatedly [6–9] that if the first infection kills the host, there is no longer any need for immunological memory. Alternatively, if the host can survive the first infection, this indicates that its immune system is capable of dealing with that particular type of infection efficiently, at least during the crucial period necessary for procreation. In evolution, such efficiency is defined by the fact that some percentage of the species survives a primary infection in the absence of a vaccine or any previous exposure. This has been well documented for the middle ages when smallpox first arrived in Europe with mortality rates of up to 80 or 90 % [13], or the more recent myxomatosis exposure of European rabbits in Australia [14].

I summarise as follows: General parameters of immunity

1. Infections by cytopathic/toxic, i.e. acutely lethal agents are controlled by innate plus adaptive resistance mechanisms [1–8]. Non-cytopathic persistent infections are non-pathogenic if an immune response is avoided or reduced [6, 9, 15]; this usually results from T cell deletion [15] and neutralizing antibody escape by mutation [16]. Remember, however, very low levels of residual infection keeps immunity active by 'infection immunity' [17–20].
2. The immune system only reacts with a timely IgM or IgG response if antigen reaches secondary lymphoid organs [21–23].
3. The best correlate of protection against infectious diseases is the pre-existing neutralizing or protective antibody level at the time of exposure to the infection [6–11]. This is best illustrated by the absolute requirement of maternal antibodies in newborns 'and infants' serum and mucosals (from mothers milk) for survival of offspring during the phase of neonatal and early childhood immuno-incompetence [8, 24].
4. B cell IgM responses against repetitive multimeric antigens or monomers plus Tlr signals are made in the absence of T help [25–28]. These IgM responses have a short half-life of $\sim$24 h, and of course have a high avidity and superb complement activation capacity. IgA production in the mucosa lamina propria is T help and secondary lymphoid organ independent [29]. For IgE, these rules are less clear: while specific IgE is strictly T help dependent, hyper-IgE is not [30, 31].
5. ELISA antibody responses usually binding at 10^5–10^6 M^{-1} represent highly frequent B cells (10^{-2}–10^{-3}) [32–34], whereas B cells producing neutralizing or protective antibodies with affinities of $>10^9$ M^{-1} in mice or $>10^{10}$ M^{-1} in humans are rare $<10^{-5}$ [35, 36].
6. Affinity maturation, i.e. improvement of binding qualities by somatic mutation is a slow process requiring weeks to months. Obviously, acutely lethal infections that are controlled immunologically must be reflected in the basic antibody (or T cell) repertoire available at the time of infection [12, 35]. In contrast, non-cytopathic infections that persist may well induce initially low affinity, non-protective antibodies that over time improve by a process of somatic mutation. An example of such a process has been documented for the neutralizing antibody response against LCMV [16, 37–39]. Usually, this process is illustrated with immunological text book

cases using nitrophenyl- (NP) or oxazolon-specific IgG responses [40, 41]. However, some of these examples are misleading, because it is usually not an improved original type of anti-NP antibody that binds better to NP. Instead, a newly selected antibody with better binding capacities comes up, and this correlates with a special groove in the binding site of this antibody, into which the small NP fits well. In this context, it is important to remember that relevant biologically active and protective antigenic sites comprise about 7–15 amino acids for contact interactions with a neutralizing antibody [26, 36]. In contrast, academic haptens such as NP are equivalent to only one amino acid. Therefore, it is not surprising that the rules derived from studying hapten-specific antibody responses do not apply to immunity [20, 34]. Also, the concept that highly specific antibodies show broad cross-reactivity is true for small phenyl-group-type haptens or ELISA-measured antibodies to plastic bound proteins, but is not true for complex but well-defined biologically relevant neutralizing antigenic sites [20, 34]. Thus, 'cross reactivity' is seen with antibody binding assays measuring low affinity/avidity antibodies, but not with serotype-specific protection assays.

7. The classical carrier hapten priming effect is valid for hapten-specific ELISA antibody responses but not for the serotype-specific neutralizing antibody responses against infectious agents. In the first case, where B cell frequencies are very high ($\sim 10^{-2}$) [32, 33, 44, 45], T help is usually limiting. In the second case of biologically relevant situations, T helper cells are always in excess and B cell frequency is limiting ($\sim 10^{-5}$) [20, 26, 34, 46]. I am not contesting the old experimental data of the classical carrier hapten experiments, I simply question their biological relevance. In view of the fact that the only correlate of protection against acute potentially lethal infections is the pre-existing level of neutralizing antibodies (or of already activated T cells) at the time of infection, text book-defined immunological memory—quicker and better [1–5]—is too slow to protect [6–8, 10].
8. Protective antibodies against serotypically defined virus or bacteria strains are non-cross-reactive by definition [6, 7, 47], and experimental- or epidemiological evaluation has revealed that cross-reactive antibodies are not protective. Therefore, attempts to raise cross-protective antibodies against Influenza viruses [48–50] or HIV clades [51–53] is useless, has failed so far, and will fail in the future. Rare viruses (or bacteria?) do not comply with this simple rule, e.g., Dengue viruses [54] exhibit defined, but partially overlapping, serotypes correlating with the not yet understood complication of hemorrhagic fever upon certain kinds of cross-reexposure.
9. Spontaneous or not intentionally induced neutralizing antibodies against acute cytopathic agents are specific and not simply a cross-reactive background [12]. They play a major role in reducing early hematogenically spreading antigen. The neutralizing titer of naïve serum against acute cytopathic virus infections is often around 1:30. Note that the difference between spontaneous and protective serum titer (i.e. >1:500) is only about 10- to 30-fold. Interestingly, against non-cytopathic viruses, such natural antibody titers usually cannot be measured [12, 16, 38, 39]. In fact, it has been found that protective neutralizing antibody responses against such persistingly non-cytopathically infecting viruses (LCMV, HIV, etc.) must undergo slow affinity maturation during which mutational escape is possible (e.g., [16, 38]).
10. Protection requires minimal affinity 10^{-9} in mice and 10^{-10}–10^{-11} in humans and concentration >1 μg/ml of protective neutralizing IgG antibody [36].
11. Plasma cells producing protective antibodies are relatively short-lived (1–5 days). Once B cells are fully induced and matured to plasma cells, they get deleted (e.g., [20, 38]). This result of course is in contrast to many studies using ELISA-type antibody responses [5, 55, 56] against haptens [1, 5, 33, 34], but also against viral antigens irrelevant for protection, such as nuclear protein [20, 55]. However, when the serotype specificity-constraints of the antibody produced by plasma cells are observed, experiments and epidemiology clearly show that only recently activated B cells become neutralizing antibody-producing plasma cells which exhibit a half-life of somewhere between 1 and 5 days [20]. This short half-life is a safe guard against autoantibody-dependent autoimmunity. Therefore, increased B cell frequencies (or memory B cells) are antigen independent, while maintenance of increased neutralizing antibody levels are antigen dependent [20].
12. Avoidance or delay of a neutralizing antibody response arises from: (1) low precursor B cell frequency [17, 38, 39, 57], (2) requirement of affinity maturation [16, 38, 39, 42], (3) immunopathological destruction of follicle organization in secondary lymphatic tissues [23], (4) variability of the protective antigen [16, 43, 58], and (5) excess T help causing hyper-IgG responses [59].
13. Sufficient levels of protective antibody must be transferred from mother to offspring via placenta (IgG) and milk (IgA/IgG) to protect the offspring during the 1–2 years after birth against acute cytopathic (childhood) infections. Attenuation of these

infections by transferred maternal antibody represents the physiological equivalent of vaccination [8, 24].

14. Epidemiologically active conditions are essential to establish active herd immunity against childhood infections [6–8, 11, 24]. It is important that offspring get exposed to epidemiologically active infection during the post-natal period where maternal antibodies are still present to promote enhanced survival and establish acquired immunity [24].
15. Successful vaccines protect humans by neutralizing antibodies via reexposure and immune complexes [6–9, 11, 60]. In contrast, we still lack efficient vaccines that maintain activated T cell responses (and/or neutralizing antibody responses) against highly variable agents for a long time as is necessary against HIV, HCV, malaria, TB, and many other infections [17, 18, 27, 57, 60].
16. Antigen dependence of protection is often masked by antigen persisting at very low levels in the herd or in the individual, often extralymphatically and sometimes in unconventional forms. This includes persistence of dormant non-replicating infections or crippled virus 'persisting' in the host [6, 7, 11, 60–64] (e.g., Herpes viruses, measles virus) or of DNA forms of conventionally integrated retroviral RNA viruses [53], or through an unconventional DNA form expressed with the help of retroviral elements in the genome of the host [65, 66]. Reexposure from outside is very commonly unrecognized, particularly for mucosal infections (reviewed in [1–9, 11, 47]).
17. Therefore, immunity represents low level antigen-driven immune responses in absence of overt immunopathology in the herd [6–9, 15]. This is best illustrated by antigen derived from reexposure from the outside or the inside of the host by immune complexes [60] (representing antigen depots of several months up to probably years). Low level persistent infections, such as herpes virus infections, TB, leprosy, or HIV constantly reboost immunity. Remember, in the absence of a TB granuloma, protective T cell immunity fades within 1–2 years, e.g., after BCG immunization, while on the other hand, too many or extensive granulomas eventually cause lethal tuberculosis.

Discussion

The term immunological memory was originally coined to seemingly understand protective immunity and to explain why and how vaccinations work. Because this definition and the experiments used to support this definition usually had nothing to do with infections (or used infectious disease antigens to study immune memory independent of protection), the apparent correlation was seriously misused, even after improved understanding of both infections and of immune responses became apparent (e.g., [6, 7, 60–64]. The present review is yet another attempt to revise generally held views and to resolve serious discrepancies between the academic ideas on immunological memory and the fact of immunity, i.e. long-term protection against lethal infections. Because words matter and because the immunological community is generally not interested in infectious diseases, the false use of 'memory' to explain protective immunity persists.

The view summarized here, of course, has serious implications not only on the general public's understanding of vaccines and public health but also has a great impact on politics and public support of science. Why do I point this out? Let us assume certain childhood infections against which we have excellent vaccines are now well controlled, but at the 'cost' of reducing epidemiologically circulating wild-type (or live vaccine strain) infections to maintain herd immunity [8, 11, 24, 47]. This is now becoming more obvious as the live-attenuated Sabin vaccine gets phased out (because of the emergence of virulent revertants) [11, 47] and is being replaced by the inactivated Salk vaccine. While until recently the live-attenuated Sabin vaccine repeatedly re-vaccinated the herd, in the future this will not happen. Of course, if there is complete elimination of polio this may not matter. However, unless this has been achieved, any new outbreak of polio would become a great potential disaster, because of exposure of adults and of newborns who possess a level of protection that is insufficient to attenuate initial infections, and therefore cannot allow for disease-free survival of virtually all members of the species. It is now being observed that very early infection with rubella or whooping cough [67, 68] has serious consequences in babies of mothers who had been vaccinated against these agents [with presumably relatively low protective (not necessarily ELISA) antibody titers] in the now altered epidemiological situation—i.e. with non-replicating vaccine agents and absent or rare re-exposure in the herd in the absence of revaccination. While these are early observations, they at least signal epidemiological conditions where circulating infections and herd immunity may become so low that general maintenance of protection by herd immunity plus vaccinations is not any longer guaranteed [24], unless revaccinations are implemented frequently for perhaps the entire life span.

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